Association between immunoscore with lymphovascular invasion and clinical staging in colorectal cancer

Raden Achmad Hussein Fachruddin\textsuperscript{1*}, Wifanto Saditya Jeo\textsuperscript{1}

ABSTRACT

Background: Despite the immunoscore benefit function as a prognostic tool in colorectal cancer, no study reports its reason. This study tried to identify the relationship between the immunoscore and the incidence of lymphovascular invasion and clinical stage in colorectal cancer patients as a mechanism for immunoscore to predict the outcome of colorectal cancer.

Methods: This is correlational with a cross-sectional study that analyzed medical record data and laboratory results. Immunoscore was collected from an independent private laboratory, and other data (gender, age, diagnosis, tumor location, lymphovascular invasion) were from medical records from the Private Hospital colorectal cancers registry. Data collection was carried out from October to December 2022. We performed data analysis with the Chi-Square statistical test with a significant p-value < 0.05.

Results: The results showed that the majority of subjects had tumor locations in the rectosigmoid (25%) and advanced stages (65%) with lymphovascular invasion findings (80%). Chi-Square statistical test shows the result of \( p = 0.025 \) and \( p = 0.64 \), respectively, lymphovascular invasion and clinical staging.

Conclusion: This study shows that the immunoscore has a dependent relationship with the incidence of lymphovascular invasion and an independent relationship with the clinical stage of TNM in colorectal cancer patients. The application and use of immunoscore are beneficial for clinicians to give suggestions and understanding to patients about their choice of therapy also outcome for the patient.

Keywords: clinical staging, colorectal cancer, immunoscore, lymphovascular invasion, prognosis.


INTRODUCTION

Colorectal cancer accounts for approximately 10% of all cancers diagnosed annually and cancer-related deaths worldwide.\textsuperscript{1,2} Colorectal cancer is also the second most common cancer diagnosed in women and the third most in men. Women have a 25% lower incidence and mortality than men, but these depend geographically, with the highest rates in most developed countries. With ongoing progress in developing countries, the worldwide incidence of colorectal cancer is expected to increase to 2.5 million new cases by 2035.\textsuperscript{1,3}

The clinical stages of colorectal cancer are classified based on the TNM classification, namely tumor extent (T), spread to nearby lymph nodes (N), and metastasis to distant organs (M).\textsuperscript{4} Also, a histopathological examination is often performed to detect lymphovascular invasion. Lymphovascular invasion is the presence of cancer cells in the blood vessels and lymph vessels. This condition is considered an early stage in spreading cancer because these two vessels are the main route for circulating cancer cells to other parts.\textsuperscript{5} All clinical staging and examination of lymphovascular invasion are used to help determine the most appropriate and prognostic management options in each colorectal cancer patient.

Surgical management is still the first choice of therapy in colorectal cases today. However, various conditions make patients unable to undergo surgical procedures, so they receive other management (radiotherapy, chemotherapy, immunotherapy).\textsuperscript{6,8} In 70-80% of colorectal cancer cases, molecular pathogenesis of colorectal cancer occurs due to disturbances in the immune system following the conventional chromosomal instability pathway. Mutations precede the pathway in adenomatous polyposis coli (APC), followed by mutations in the Kirsten Rat Sarcoma viral oncogene homolog (KRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and mothers against decapentaplegic homolog 4 (SMAD4) as well as loss of heterozygosity of chromosome 18 (LOH 18q) and mutation of the tumor gene protein 53 (TP53).\textsuperscript{6} This shows that cancer cells, in general, have the property of suppressing the response and work of the body’s immune cells. On the other hand, various studies have shown a correlation between a high number of tumor-infiltrating CD8+ T lymphocytes (TIL) with a better prognosis in colorectal cancer patients.\textsuperscript{7}

Pagès F et al. confirmed that high numbers of tumor-infiltrating memory T cells in colorectal cancer tissue are associated with no tumor metastases finding, preventing tumor development, and better survival.\textsuperscript{8} The study by Malka

Open access: www.balimedicaljournal.org

1\textsuperscript{Digestive Surgery Division, Surgery Department, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia;}

*Corresponding author: Raden Achmad Hussein Fachruddin; Digestive Surgery Division, Surgery Department, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia; Radenhufach@gmail.com

Received: 2022-12-26
Accepted: 2023-03-22
Published: 2023-04-04

Published: 2023-04-04
Until now, the most consistent immune parameters that have a relationship with prognosis in colorectal cancer patients are T cells (CD3+), cytotoxic T cells (CD8+), and memory T cells (CD45RO+). There are various immune system calculation systems, one of which is Immunoscore®, calculated based on immunohistochemical examination with a digital image analysis system for CD3+ and CD8+ lymphocyte density in the center and tumor invasive margins. However, until now, it is still unclear whether there is a direct link between a better prognosis and the activity of the patient’s immune system. This study aims to study the relationship between the immunoscore and the incidence of lymphovascular invasion and clinical stage in colorectal cancer, which has not been done before.

**METHODS**

**Study Population**

This cross-sectional study analyzed medical record data, and laboratory results from all colorectal cancer patients enrolled in the digestive division of the Surgery Department at Siloam Hospital Kebon Jeruk. Data collection was carried out from October 2022 to December 2022. The inclusion criteria included all patients aged over 18 years who were diagnosed with colorectal cancer based on clinical and histopathological examination and an immunoscore examination. Immunoscore analysis was conducted using immunohistochemical techniques at the “Medis Khusus Utama: KALGen Innolab” Laboratory at Jend. Ahmad Yani Street No.2, Jakarta, Indonesia. Subjects who had an immunodeficiency disease or received immune system modification therapy (steroids, immunosuppression, immunomodulators) also did not have complete data were excluded. Immunodeficiency diseases include acquired immunodeficiency syndrome (AIDS), leukemia, complex immune disease, viral hepatitis, multiple myeloma, chronic diseases (diabetes, renal failure), ataxia-telangiectasia, complement deficiency, DiGeorge syndrome, hypogammaglobulinemia, Job’s syndrome, and leukocyte adhesion defects.

The sample size calculation used the ratio formula for the proportions of two groups, which resulted in a total of 80 subjects divided into two groups (high and low immunoscores) of 40 subjects each. Every patient diagnosed with colorectal cancer with an immunoscore examination will be entered consecutively until the required sample size is reached. Clinical staging is determined based on CT scans and biopsy investigations, which are grouped into early stages (I, II) and advanced stages (III, IV) according to the 2018 AJCC/UICC TNM classification system.9,10 Meanwhile, lymphovascular invasion is defined as tumor cells in the endothelial or damage to the walls of blood vessels and lymphatics due to tumor cells based on the results of pathology anatomy examination. Before the OncoPanel-Colorectal examination, all subjects were diagnosed using the applicable standard histology pathology.

Determining the tumor’s location was based on a CT scan examination and continued with confirmation of histopathological biopsy examination to obtain data on the patient’s lymphovascular invasion and clinical stage. An immunoscore examination was performed using a formalin-fixed paraffin-embedded (FFPE) specimen stained with hematoxylin-eosin. This study involved five examinators in processing each sample and evaluated the immunoscore digitally using a calibrated machine. All data have a low risk of bias due to being managed by Indonesia’s legal and accredited private laboratory. An international standardized was processed to calculate the density of CD3+ and CD8+ at the tumor center and edges. The results of the immunoscore calculation will be divided into two categories: high (I3, I4) and low (I0, I1, I2). This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (No: KET-1260/UN2.F1/ETIK/PPM.00.02/2022).

**Data collection and analysis**

All registered patients at the digestive division of the Surgery Department at Siloam Hospital Kebon Jeruk medical records with immunoscore examination are consecutively collected for this study. Data from medical records included gender, age, and location of cancer occurrence and ensured that patients met the exclusion criteria without comorbidities. Data regarding the clinical stage of TNM and information on lymphovascular invasion were obtained through imaging and anatomical pathology results. In comparison, the immunoscore was obtained from laboratory results by KALGen Innolab. All data was taken and tidied up in Microsoft Excel before being analyzed using SPSS software version 27 for Mac.

All variables were analyzed to ensure distribution and percentage so that the two groups had no different demographic characteristics. Categorical data (gender, tumor location, clinical stage, and lymphovascular invasion) are presented in tables, and numerical data (age) is shown in mean (standard deviation) form. Age data were tested for normality using the Kolmogorov-Smirnov test. Analysis of the independent variable (immunoscore) with the dependent variable (lymphovascular invasion, clinical stage), both are categorical data, so using the Chi-Square test.

**RESULTS**

We included 80 subjects in this study, with 40 subjects for each group, the high and low immunoscore groups. The two groups did not have statistically significant differences in terms of gender (p=0.37), age (p=0.45), and tumor location (0.58). As in Table 1, the number of men (n=38) and women (n=42) is balanced, with 21 men in the high immunoscore group and 23 women in the low immunoscore group. The age showed a normal distribution (p=0.63) with an overall mean value of 55.69±13.23 years. The majority of subjects had tumor locations in the rectosigmoid (n=20), and rectum (n=19) followed respectively by a sigmoid colon (n=11), ascending (n=10), descending (n=9), transverse (n=7), and cecum (n=4).

The majority of the data were from advanced stages based on histopathological examination (65%) and were relatively distributed between the high (51.90%) and low (48.10%) immunoscore groups.
The statistical analysis results between the clinical stage and the immunoscore did not show a significant relationship (p = 0.64) (Table 2).

As many as 64 subjects (80.00%) found lymphovascular invasion and 56.25% were in the low immunoscore group, as shown in Table 3. Sixteen subjects (20.00%) did not find lymphovascular invasion, and 75.00% were in the high immunoscore group. Based on statistical analysis, there was a significant relationship (p=0.025) between immunoscore status and findings of lymphovascular invasion with an odds ratio of 0.259, which means that patients in the high immunoscore group have a protective factor against lymphovascular invasion.

**DISCUSSION**

The tumor, nodal, metastatic (TNM) cancer classification system has been in everyday use for more than 80 years. Unfortunately, the TNM system does not sufficiently inform prognosis and clinical outcomes among patients with histologically the same tumor stage. The TNM system does not consider the immune response and only focuses on indicators routinely used, there is no significant correlation between them. In this finding, there was also no significant difference between gender, age, and tumor location by immunoscore. This finding is in line with the study of Pagès F et al. and Church D et al., who concluded that the immunoscore was independent of the clinical stage of TNM. In this study, patients with a high immunoscore had a lower risk (OR = 0.256) of developing lymphovascular invasion. This finding may explain why patients with a high immunoscore have a better prognosis than patients who are found to have minimal lymphocytic infiltration of tumor cells. The incidence of lymphovascular invasion is a poor predictor of the outcome of all cancer patients, including colorectal cancer.19,20 From this study, lymphocyte infiltration into tumor cells was found to be a protective factor against lymphovascular invasion. This outcome aligns with a study by Pagès F et al., which showed a significant association between lymphovascular invasion and immunoscore.17 Although it is known that the immunoscore and clinical stage of TNM is prognostic indicators routinely used, there is no significant correlation between them. In this finding, there was also no significant difference between gender, age, and tumor location by immunoscore. This finding is in line with the study of Pagès F et al. and Church D et al., who concluded that the immunoscore was independent of age, sex, tumor stage, node stage, tumor development.

The presence of lymphocytes infiltrating primitive tumors can increase the prognosis of overall survival (OS) and disease-free survival (DFS) because it correlates directly with micro-invasive status, and CD8+ T cells are in the center of the tumor suggests a vital role in the immune response.11,16 The study of Pagès F et al. and Mlecnick B et al. demonstrated that Immunoscore significantly predicts time to recurrence (TTR), disease-free and overall survival, and populations at high and low risk of recurrence.17,18 The study of Mlecnick B et al. showed that a high Immunoscore significantly has a better TTR and survival rate.18 However, in these studies, it is not yet known how the exact mechanism of lymphocyte infiltration can affect the outcome of each colorectal patient with the same TNM stage.

This study showed that the immunoscore had a significant dependent relationship to lymphovascular invasion while independent of the clinical stage of TNM. In this study, patients with a high immunoscore had a lower risk (OR = 0.256) of developing lymphovascular invasion. This finding may explain why patients with a high immunoscore have a better prognosis than patients who are found to have minimal lymphocytic infiltration of tumor cells. The incidence of lymphovascular invasion is a poor predictor of the outcome of all cancer patients, including colorectal cancer.19,20 From this study, lymphocyte infiltration into tumor cells was found to be a protective factor against lymphovascular invasion. This outcome aligns with a study by Pagès F et al., which showed a significant association between lymphovascular invasion and immunoscore.17 Although it is known that the immunoscore and clinical stage of TNM is prognostic indicators routinely used, there is no significant correlation between them. In this finding, there was also no significant difference between gender, age, and tumor location by immunoscore. This finding is in line with the study of Pagès F et al. and Church D et al., who concluded that the immunoscore was independent of age, sex, tumor stage, node stage, tumor development.

### Table 1. Demographic characteristics based on immunoscore group high and low.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immunoscore High (n = 40)</th>
<th>Immunoscore Low (n = 40)</th>
<th>PR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>17</td>
<td>1.22</td>
<td>0.79-1.89</td>
<td>0.37</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>23</td>
<td>0.82</td>
<td>0.52-1.28</td>
<td></td>
</tr>
<tr>
<td>Age (Mean±SD)</td>
<td>56.83±13.54</td>
<td>54.55±13.10</td>
<td>2.28</td>
<td>-3.65-8.21</td>
<td>0.45</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caecum</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascendens</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transverse</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Descendents</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>6</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>9</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>9</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PR: Prevalence Ratio; CI: Confidence Interval; *Statistically significant if p-value less than 0.05.

### Table 2. Association between clinical staging with immunoscore group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immunoscore High (n = 40)</th>
<th>Immunoscore Low (n = 40)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>13</td>
<td>15</td>
<td>0.80</td>
<td>0.32-2.02</td>
<td>0.64</td>
</tr>
<tr>
<td>Advanced</td>
<td>27</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: Odds Ratio; CI: Confidence Interval; *Statistically significant if p-value less than 0.05.

### Table 3. Association between lymphovascular invasion with immunoscore group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immunoscore High (n = 40)</th>
<th>Immunoscore Low (n = 40)</th>
<th>OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>36</td>
<td>0.259</td>
<td>0.08-0.025*</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>4</td>
<td>0.891</td>
<td></td>
</tr>
</tbody>
</table>

OR: Odds Ratio; CI: Confidence Interval; *Statistically significant if p-value less than 0.05.
Immunoscore has been declared valid in North America, Europe, and Asia to compare low and high scores in recurrence-free survival. However, it has only been shown in univariate analysis. The International Immunoscore Project was established to develop and evaluate a standardized approach to testing consensus immune biomarkers.25–24 In this study, the immunoscore examination followed the applicable provisions using resected tumor tissue, then fixed with formalin-fixed paraffin-embedded (FFPE) and stained with hematoxylin-eosin. After that, the specimens were divided into 4µm sized samples, especially in the central part of the tumor and invasive edges, then placed in glass slides. The paraffin flakes in the preparation were then transferred to a tube, deparaffinized, and centrifuged. Next, it was washed with 70% alcohol and processed for DNA extraction using a diluted kit and stored at a specific temperature. The tissue specimens were then analyzed for the number of copies using PCR and calculated using the software. The preparations that have been stained are then scanned for the digital coloring process. Next, the densities between the positive and stained cells were calculated using digital pathology soft tissue. The immune score was calculated based on the CD3+ and CD8+ densities in two tumor areas: the central part of the tumor and the invasive edge.23

Although the mechanism by which the immunoscore can predict prognostic outcomes is still unknown, various studies continue to report the superior ability of the immunoscore in predictors of colorectal cancer patients. The study of Trabelsi M et al. evaluated the value of the immune score in colorectal cancer patients. The study complied with the planned research methodology. Still, there were some limitations, one of which was that this study only carried out secondary sampling and did not report outcomes for each sample. On the other hand, secondary data collection indirectly reduces the possibility of bias occurring in the research results because different parties carry out the validation and analysis. Due to limited resources, this study did not follow every subject, so it did not receive output data from each subject. In addition, because this study was a cross-sectional study that collected secondary data and the available data was limited, no selection was made for different comorbidities other than immunosuppressant or immunomodulatory medication or systemic disease. Therefore, we propose to conduct a future cohort study regarding the effectiveness and cost comparison of the implementation of the immunoscore in determining the prognostic value and prediction of chemotherapy response in cancer patients in Indonesia. In addition, the study can be supplemented by analyzing other factors that might affect the immunoscore result.

CONCLUSION

This study shows that the immunoscore has a dependent relationship with the incidence of lymphovascular invasion. In contrast, an independent relationship was found with the clinical stage of TNM in colorectal cancer patients. Although it is not yet known what mechanisms and factors can influence the immunoscore test, its application and use in clinical situations can help clinicians and patients understand the prognosis and the choice of therapy that can produce the best outcome for the patient.

CONFLICT OF INTEREST

All the authors state no conflicts of interest in this manuscript. The authors are responsible for all financing without grant or external funding sources.

ETHICAL CONSIDERATIONS

This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (No: KET-1260/UN2.F1/ETIK/PPM.00.02/2022).

FUNDING

None.
AUTHOR CONTRIBUTIONS

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study's results were interpreted upon publication.

REFERENCES


